

## Amniocentesis and Chorionic Villus Sampling

LEE P. SHULMAN, MD, and SHERMAN ELIAS, MD, *Memphis, Tennessee*

**Amniocentesis and chorionic villus sampling have been shown through prospective, multicenter trials to be safe and effective methods of prenatal diagnosis; accordingly, a knowledge of these tests is important for those physicians who care for women during their childbearing years. We review the indications, techniques, safety, accuracy, and efficacy of amniocentesis and chorionic villus sampling and compare the advantages and disadvantages of each diagnostic test. This review should enable physicians to provide appropriate counseling and information to women at increased risk for fetal abnormalities detectable by either of these procedures.**

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**I**nvasive prenatal diagnosis of fetal abnormalities has become an integral part of obstetrics and perinatal medicine. Midtrimester amniocentesis has traditionally been the most common technique used for invasive prenatal diagnosis; however, the desire for first-trimester prenatal diagnosis has led to the development and eventual integration of chorionic villus sampling into the invasive prenatal diagnostic repertoire. In addition, the desire to diagnose prenatal abnormalities earlier in pregnancy has led to the increasing use of amniocentesis in the first and early second trimesters.

We review the uses of amniocentesis, both traditional and early, and chorionic villus sampling for the prenatal diagnosis of fetal abnormalities in the first and second trimesters of pregnancy. Because these two diagnostic tests have become integral to obstetric practice in the United States, familiarity with the techniques, risks, and limitations of these tests is critical to those physicians who care for women of childbearing age.

### Amniocentesis

Over the past two decades, second-trimester amniocentesis has become a standard procedure for the diagnosis of fetal genetic abnormalities. Cytogenetic, enzymatic, and DNA analyses can be done on cells obtained from amniotic fluid. In addition, levels of  $\alpha$ -fetoprotein (AFP) and acetylcholinesterase (AChE) in the amniotic fluid can be measured to diagnose neural tube defects (such as spina bifida and anencephaly) and anterior abdominal wall defects (for example, omphalocele and gastroschisis) prenatally. Thus, amniocentesis is applicable for the prenatal diagnosis of many fetal abnormalities.

### Technique

Conventional genetic amniocentesis is usually performed between 14 and 20 weeks' gestation ("menstrual weeks") to evaluate a fetus for chromosome abnormalities, neural tube defects, and other detectable genetic and acquired disorders (Table 1). An ultrasound examination should be done immediately before amniocentesis to evaluate fetal number and viability, confirm gestational age, assess placental location, and estimate amniotic fluid volume. We routinely perform a detailed fetal anatomic survey. In addition, a complete counseling session should precede amniocentesis or any other prenatal diagnostic test; indications for testing and the risks, benefits, and limitations of the prenatal test should be reviewed in detail and in language understandable by the patient.

Once the preoperative ultrasound examination and counseling are completed, a site is selected for inserting the needle into the amniotic cavity. Close attention should be paid to the location of the small bowel and bladder to avoid puncturing them. The needle insertion site is then cleansed with an iodine-based solution and draped with sterile towels. Some obstetricians use a local anesthetic, such as 5 ml of a 1% solution of lidocaine hydrochloride, before inserting the needle, but this is usually unnecessary.

We prefer a 22-gauge spinal needle and recommend no larger than a 20-gauge needle to do amniocentesis. The needle is inserted transabdominally into the amniotic cavity under continuous ultrasonographic guidance (Figure 1).<sup>1</sup> The needle should be inserted with one smooth, continuous motion until the needle tip is within the amniotic cavity. In some cases, a gentle "pop" can be felt by

From the Division of Reproductive Genetics, Department of Obstetrics and Gynecology, University of Tennessee, Memphis, College of Medicine.

Reprint requests to Lee P. Shulman, MD, Dept of Obstetrics and Gynecology, University of Tennessee, Memphis, 853 Jefferson Ave, Rm E-102, Memphis, TN 38103-2896.

**ABBREVIATIONS USED IN TEXT**

AChE = acetylcholinesterase

AFP =  $\alpha$ -fetoprotein

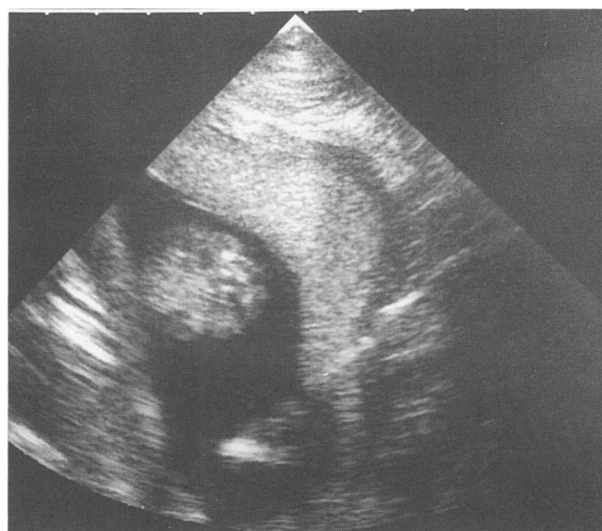
CVS = chorionic villus sampling

the obstetrician when the needle tip enters the amniotic cavity; however, this sensation does not always signal entry into the amniotic cavity, especially when the amniocentesis is performed before 14 weeks' gestation. Once the needle tip is in the amniotic cavity, the needle stylet is removed and a syringe is attached to the needle hub (Figure 2).<sup>2</sup>

Approximately 1 ml of amniotic fluid is aspirated initially; the syringe and this small amount of fluid are discarded to reduce the risk of specimen contamination by maternal cells. For second-trimester amniocentesis done between 14 and 20 weeks inclusive (that is, conventional amniocentesis), 20 to 30 ml of amniotic fluid is usually aspirated. Failure to aspirate amniotic fluid is most commonly due to membrane "tenting." When this occurs, the needle appears by ultrasonography to be within the amniotic cavity, but the needle tip has not penetrated the amniotic sac. This occurs more frequently with amniocentesis performed before 14 weeks' gestation because amnion and chorion are usually not fused early in gestation. Other causes of aspiration failure include tissue blockage of the needle tip and juxtaposition of the needle tip to fetal structures or membranes.

Once fluid aspiration is completed and the needle has been removed from the amniotic cavity, a cursory ultrasound examination is done to confirm fetal cardiac activity and to look for evidence of bleeding from the placenta, umbilical cord, or fetus. We administer 300  $\mu$ g of Rh immune globulin to all unsensitized, Rh-negative patients, regardless of whether the placenta has been traversed by the needle. The patient may resume all normal activities following amniocentesis, but common sense dictates that strenuous activity be avoided for several days. The patient is instructed to notify the physician of persistent uterine cramping, bleeding or leakage of amniotic fluid from the vagina, or fever.<sup>1</sup>

Amniocentesis can usually be done in cases of multi-



**Figure 1.**—In an ultrasonogram of an amniocentesis done at 15.1 weeks' gestation, the needle tip is visualized within the amniotic cavity (from Shulman et al').

ple gestations<sup>1,3,4</sup>; separate sacs must exist for each fetus, and each must contain adequate amniotic fluid. After fluid is aspirated from the first sac, 2 to 3 ml of indigo carmine (diluted 1:10 in bacteriostatic water) is injected before the needle is withdrawn. A second amniocentesis is then done, with a fresh needle insertion site chosen to direct the needle into the second amniotic sac. The aspiration of clear amniotic fluid confirms that the second sac has been entered. An experienced physician can successfully perform twin amniocenteses in 95% of cases, with ostensibly no more procedure-related risks than in single pregnancies; of course, risks for fetal morbidity and mortality are greater in multiple gestations than in single pregnancies. Each additional fetus can be evaluated similarly by injecting indigo carmine solution into successive amniotic sacs.

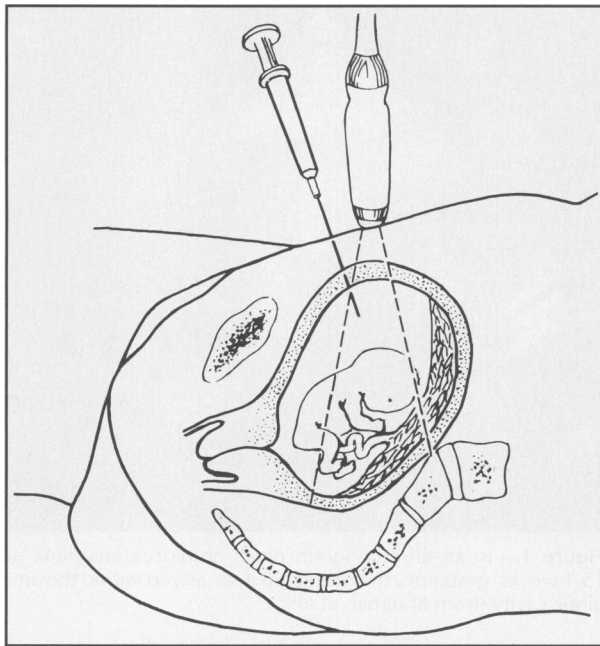
### Safety

The safety of genetic amniocentesis has been addressed by several large collaborative studies. The original trial was sponsored by the National Institute of Child Health and Development<sup>5</sup>; of 1,040 women undergoing amniocentesis, 3.5% experienced fetal loss after amniocentesis compared with 3.2% of concurrent controls (992 patients). This small difference was not statistically significant. In the United Kingdom, a collaborative trial revealed that the loss rate after amniocentesis was significantly higher than in controls (2.6% for the amniocentesis group compared with 1% for controls).<sup>6</sup> A common indication for amniocentesis in the United Kingdom group, however, was an elevated maternal serum AFP level, a factor associated with increased fetal mortality. In a later analysis, after subjects undergoing amniocentesis for that reason were excluded, the difference between subject and control groups was reduced to less than 1%. Nonetheless, this difference remained significant.

High-resolution ultrasonography was not employed in

**TABLE 1.**—*Indications for Offering Invasive Prenatal Diagnosis*

Increased risk for fetal chromosome abnormalities
Advanced maternal age ( $\geq 35$ yrs at time of delivery)
Previous offspring with chromosome abnormality
Parental chromosome abnormality
Balanced parental chromosome rearrangement
Miscarriages (3 or more)
Fetal structural defects (cystic hygroma)
Increased risk for mendelian disorders detectable by molecular biologic techniques (sickle cell anemia, cystic fibrosis)
Increased risk for mendelian disorders detectable by enzyme assays (Tay-Sachs disease)
Increased risk for polygenic or multifactorial conditions detectable by amniotic fluid analyses (neural tube defects, anterior abdominal wall defects)



**Figure 2.**—The diagram illustrates the proper amniocentesis procedure (from Simpson and Elias<sup>2</sup>).

either the American or British collaborative trial; in fact, ultrasonography was sometimes not used at all. A possible explanation for the ostensible failure of high-quality ultrasound studies to decrease the pregnancy loss rate is that loss rates in the original US study, specifically in the control group, were spuriously high. Indeed, the reported loss rate after 16 weeks in controls (3.2%) in the US trial was actually closer to that expected at 8 to 9 weeks' gestation,<sup>7</sup> although variables such as differences in maternal age make precise comparisons difficult. Moreover, some obstetricians participating in the American and British studies were relatively inexperienced with the procedure. Therefore, contemporary obstetricians may assume that the actual increase in loss rate contributed by amniocentesis is less than the 0.5% usually counseled. In a prospective randomized study involving 30- to 34-year-old Danish women, however, the loss rate was 1.7% in the amniocentesis group and only 0.7% in the control group ( $P < .05$ ).<sup>8</sup> The obstetricians involved were highly experienced and used high-resolution concurrent ultrasonography. Indeed, to date, no large studies have shown the increase in loss rate to be less than 0.5%. We therefore recommend counseling patients that the increased risk of fetal mortality attributable to amniocentesis may be as high as 0.5%. Finally, we counsel patients that the risks of serious maternal complications or fetal injuries are remote but do exist.

#### *Accuracy*

The analysis of amniotic fluid, as well as that of chorionic villi or fetal tissue, entails difficulties that need to be recognized by obstetricians caring for patients who undergo invasive prenatal diagnostic tests. First, cells obtained by amniocentesis may not grow, or growth may be

insufficient to obtain metaphases for cytogenetic analysis. Amniotic cell cultures are usually successful; both Canadian<sup>9</sup> and American<sup>10</sup> collaborative trials comparing chorionic villus sampling (CVS) and amniocentesis revealed the failure to obtain cytogenetic results for patients undergoing amniocentesis to be uncommon (0.1% in the Canadian study and 0.9% in the American study).

Second, maternal cells may be inadvertently included in the specimen, thereby creating the possibility of an incorrect diagnosis. This source of possible error is of greater concern with CVS and percutaneous umbilical blood sampling; in theory, discarding the syringe containing the first milliliter of aspirated amniotic fluid should reduce the chance of maternal cell contamination.

A third source of error involves chromosome abnormalities that are not representative of fetal complement. Such chromosome abnormalities may arise in culture and should be suspected whenever they are restricted to only one of the several culture flasks or clones started from a single amniotic fluid specimen. In fact, cells containing at least one additional structurally normal chromosome are detected in 1% to 2% of all amniotic fluid specimens.<sup>11,12</sup> When such cells are confined to a single culture or clone, the phenomenon is termed pseudomosaicism; when they are found in more than one flask or clone, the phenomenon is termed true mosaicism. True mosaicism is found in 0.25% of amniotic fluid specimens, and true mosaicism is confirmed by studies of the abortus or neonate in 70% to 80% of cases.<sup>11</sup> Although pseudomosaicism is not associated with an increased risk for fetal morbidity or mortality, true mosaicism is associated with an increased risk of phenotypic and developmental abnormalities.

A fourth possible problem is that some phenotypes are difficult to predict from the chromosome complement. This is especially the case when an apparently balanced translocation, small inversion, or small supernumerary chromosome is identified. If one of the phenotypically normal parents has the same chromosome aberration, reassurance is generally appropriate. Apparently balanced *de novo* structural abnormalities, such as chromosome translocations and supernumerary chromosomes, are associated with about a 10% risk of phenotypic abnormalities.<sup>13</sup>

Patients must therefore be made aware that although laboratory failure and cytogenetic discrepancies are now uncommon in amniocentesis, they do occur and may lead the physician to recommend either a second amniocentesis or a different diagnostic test, such as percutaneous umbilical blood sampling, to further evaluate the fetal state.

#### *Early Amniocentesis*

The desire to provide invasive prenatal diagnosis in the first trimester of pregnancy led to the development of chorionic villus sampling, a procedure found to be both safe and reliable. Many medical centers, however, do not have the capabilities to do CVS and have begun to investigate the use of amniocentesis before 15 weeks' gestation. Also, a prenatal diagnostic procedure should be available to women presenting after the 12th gestational

week, the closing time for acceptance into most CVS programs, but before the 15th gestational week when conventional amniocentesis can be done. No set definition of early amniocentesis exists as yet; many researchers define early amniocentesis as a procedure performed before the 15th gestational week. At our institution, those procedures done before the 14th gestational week are considered "early."

Early amniocentesis is done in a manner similar to traditional amniocentesis, except that less fluid is removed, usually 1 ml for each completed week of gestation.<sup>1</sup> Some authors have described difficulty in obtaining the amniotic fluid specimen at less than 13 weeks' gestation; this presumably results from membrane tenting, the chance of which is greater before 14 weeks' gestation.

Data from several studies suggest that early amniocentesis is a promising technique for first-trimester prenatal diagnosis. Benacerraf and colleagues reported on 100 consecutive patients undergoing amniocentesis between 11 and 14 weeks' gestation.<sup>14</sup> Among 94 pregnancies allowed to continue, "all . . . were progressing normally at follow-up, which occurred at delivery or 1 month or more after the procedure."<sup>14(p710)</sup> Nevin and associates reported on 222 early amniocenteses; 60% were done at 14 weeks' gestation, 27% at 13 weeks, and 11% at 12 weeks.<sup>15</sup> The postprocedure abortion rate was 1.4%. Penso and Frigoletto reported on 407 women undergoing amniocentesis between 11 and 14 weeks and found a loss rate within four weeks of the procedure to be 2.3%, with another 1.6% loss thereafter.<sup>16</sup> In 1992 Hanson and co-workers reported a total postprocedural loss rate of 3.4% among 936 women undergoing amniocentesis at 12.8 weeks' gestation or earlier.<sup>17</sup> In the same year, Henry and Miller presented information on 1,805 early amniocenteses, 35% of which were done at 14 weeks' gestation or earlier.<sup>18</sup> The total loss rate in this study was 0.6%; the loss rate in procedures performed between 14.0 and 14.9 weeks was similar to the loss rate in procedures performed earlier than 14.0 weeks.

These reports suggest an increasing use of early amniocentesis for prenatal diagnosis. Moreover, the impression among some obstetricians is that early amniocentesis involves no special techniques or concerns about safety or accuracy. Henry and associates in their report of 55 cases declared that early amniocentesis "requires no new techniques in counseling, clinical procedure or laboratory analysis."<sup>19(pA219)</sup> Sandstrom and colleagues wrote that "early amniocentesis is an alternative method of prenatal diagnosis."<sup>20(pA385)</sup> Burton and co-workers described a 68% increase in early specimens received by their laboratory after informing local obstetricians of the availability of the laboratory analysis and the usefulness of this service<sup>21</sup>; several reference laboratories and prenatal diagnostic services now advertise early amniocentesis in commercial brochures. Early amniocentesis apparently is being offered as a routine prenatal diagnostic test, with patients being informed that the safety and accuracy of the procedure are equivalent to those of traditional amniocentesis.

We do not think reported data as yet bear out the contention that early and conventional amniocentesis are equal in safety and accuracy. Fetal loss rates following early amniocentesis and the reliability of the procedure need to be evaluated more rigorously. Most reports and abstracts provide estimates of fetal loss rates based only on pregnancies in progress, with little reference to a delivered cohort of patients. A report by Dunn and Godmilow<sup>22</sup> was especially instructive in this regard because initially they were advocates of early amniocentesis.<sup>23,24</sup> In their series, the loss rate before 28 weeks' gestation was 1.9% after CVS, 4.2% after early amniocentesis, and 1.1% after traditional amniocentesis ( $P < .001$ ). Burton and associates<sup>21</sup> and Crandall and colleagues<sup>25</sup> have reported diagnostic discrepancies between certain tests done on amniotic fluid specimens obtained in the first and early second trimesters. In addition, Wathen and co-workers found that the rapidly changing levels of AFP from 8 to 10 weeks' gestation made such analyses impracticable for prenatal diagnosis.<sup>26</sup> Further studies are needed to assess the efficacy of AFP, AChE, and other biochemical marker studies in amniotic fluid specimens obtained from early amniocentesis procedures. Overall, early amniocentesis cannot yet be assumed to be as safe or effective as traditional amniocentesis, although initial data suggest it may eventually be proved as safe and reliable as conventional amniocentesis or CVS.

### Chorionic Villus Sampling

Second-trimester amniocentesis has traditionally been the most common invasive technique for the prenatal diagnosis of genetic disorders. A safe and reliable test for first-trimester prenatal diagnosis has long been sought. Such a technique would be desirable for several reasons. Patient privacy would be protected because testing would be done at a stage when ostensible signs of pregnancy are few. First-trimester prenatal diagnosis would permit a woman who has been diagnosed as carrying a fetus with an abnormality to undergo first-trimester pregnancy termination, a procedure associated with low morbidity and substantial psychological benefit. Early diagnosis may be necessary for fetal treatment, as is demonstrated by the prevention of female pseudohermaphroditism in the treatment of fetal 21-hydroxylase deficiency with dexamethasone administered to the mother.

Chorionic villus sampling is now recognized as a safe and reliable method for first-trimester prenatal diagnosis. In this section we review techniques, safety, and efficacy of first-trimester CVS and the increasing use of the technique for prenatal diagnosis later in pregnancy.

#### Technique

Chorionic villus sampling for first-trimester prenatal diagnosis is usually done between 9.5 and 12.5 weeks' gestation. The indications for this procedure are essentially the same as those for amniocentesis, except that some fetal abnormalities, such as neural tube defects and anterior abdominal wall defects, are amenable to prenatal diagnosis only by analysis of amniotic fluid; patients at

increased risk for such abnormalities are not candidates for CVS (Table 1).

As with amniocentesis, a complete counseling session should precede the sampling procedure. In addition, a detailed ultrasound examination should be done before sampling to assess fetal viability, number, and gestational age and placental location. Also necessary are results of an indirect Coombs' test and determination of the patient's ABO and Rh status. Maternal sensitization is a relative contraindication to CVS, and all Rh-negative women whose partners are Rh positive or who are unaware of their blood type should receive Rh immune globulin after the sampling procedure.

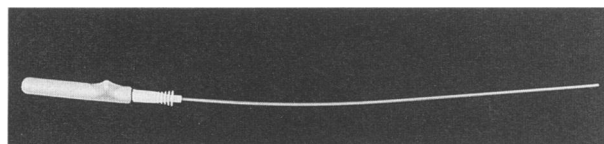
The selection of an appropriate approach is based primarily on placental location, but certain conditions may preclude a given approach. For example, uterine leiomyomata or intervening intra-abdominal structures, such as the small bowel, may preclude transabdominal CVS, whereas active cervicovaginal disease, such as herpes or chronic cervicitis, may preclude transcervical and transvaginal approaches. At our institution, this technique is offered to women with multiple gestations only if placentas are clearly separate and individually accessible.

After sampling, fetal heart activity is verified by ultrasonography and all patients are monitored for any untoward effects for about 30 minutes. Rh immune globulin (300  $\mu$ g at our institution) is administered to appropriate patients, and all patients are encouraged to consider maternal serum AFP screening at 15 to 20 weeks' gestation.<sup>1</sup>

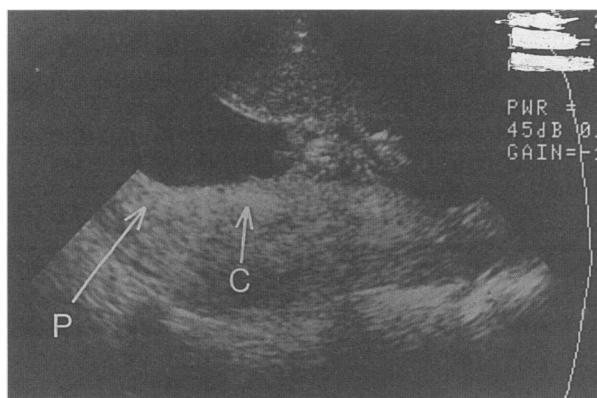
**Transcervical approach.** For transcervical CVS, patients are placed in the lithotomy position. After a speculum is placed, the vagina is cleansed with an iodine-based solution. Under continuous ultrasound guidance, a plastic catheter with an inner, malleable metal obturator (Figure 3) is introduced transcervically into the placenta (Figure 4). The optimal catheter placement is along the long axis of the placenta and away from the gestational sac and myometrium. Once the catheter is properly placed, the obturator is removed and the hub of the catheter is attached to a 20- or 30-ml syringe (Figure 5) that contains 4 to 5 ml of cytogenetic transport medium.<sup>2</sup>

Chorionic villi are obtained by 10 to 15 rapid aspirations of the syringe plunger to 20 or 30 ml negative pressure. The visualization of blood slowly moving up the catheter is an indication of successful sampling. When the procedure is complete, the catheter is removed under continuous negative pressure. An adequate specimen is approximately 5 to 8 mg, with optimal specimens weighing 15 to 25 mg.<sup>1</sup>

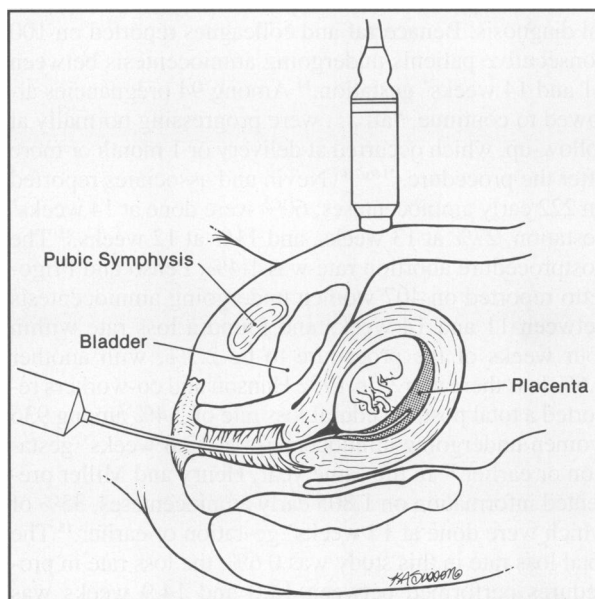
**Transabdominal approach.** For transabdominal CVS, the patient is placed in the supine position. An appropri-



**Figure 3.**—An aspiration catheter is used for transcervical chorionic villus sampling (from Shulman et al<sup>1</sup>).



**Figure 4.**—An ultrasonogram shows transcervical chorionic villus sampling. The catheter (C) is within the placenta (P).

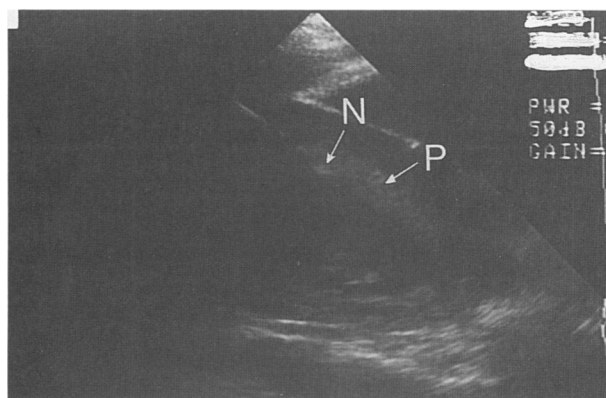


**Figure 5.**—The transcervical chorionic villus sampling procedure is illustrated (from Simpson and Elias<sup>2</sup>).

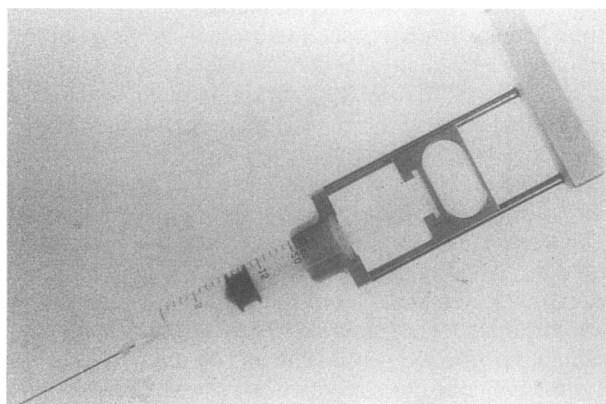
ate needle insertion site is selected during ultrasonographic examination before the procedure; in addition, close attention must be given to the location of the small bowel, which should always be avoided. Once a site is selected, the overlying skin is infiltrated with 5 ml of 1% lidocaine solution and then cleansed with an iodine-based solution.

A 19-gauge spinal needle is inserted through the maternal abdominal and uterine wall and into the placenta under continuous ultrasound guidance. The needle tip is then guided into the long axis of the placenta (Figure 6). Once the needle tip is in place, the stylet is withdrawn and a 20- or 30-ml syringe with 4 to 5 ml of transport medium is attached to the needle hub. Transabdominal aspiration is performed with the syringe attached to an aspiration device (Figure 7) to facilitate villus aspiration.<sup>27</sup> Chorionic villi are obtained in a manner similar to transcervical sampling, although the needle tip can be redirected within the placenta to obtain specimens from various sites and





**Figure 6.**—In this ultrasonogram of transabdominal chorionic villus sampling, the needle tip (N) is within the placenta (P).



**Figure 7.**—An aspiration device is used to facilitate chorionic villus aspiration for transabdominal sampling (from Elias et al<sup>27</sup>).

further facilitate aspiration (Figure 8). Optimal specimens usually weigh 10 to 20 mg.<sup>1</sup>

**Transvaginal approach.** Transvaginal sampling is usually reserved for women presenting with a retroverted, retroflexed uterus with a posterior or fundal-implanted placenta. In this situation, neither the transcervical nor the transabdominal technique is possible. Patients are prepared in a manner similar to that for transcervical CVS. After the vagina is cleansed with an iodine-based solution, the posterior wall of the vagina is anesthetized with 2 to 5 ml of a 1% lidocaine solution. During the placement of lidocaine, transabdominal ultrasonography is used to follow the needle tip within the vaginal mucosa and assess whether continuous visualization of the aspiration needle is feasible (Figure 9). If not, transvaginal CVS using endovaginal guidance may be considered, or prenatal diagnosis may be deferred until the second trimester. At our center, transvaginal sampling is offered to about 4% of women desiring first-trimester CVS; less than 1% of women who desire some form of CVS are unable to undergo the procedure because of technical considerations.

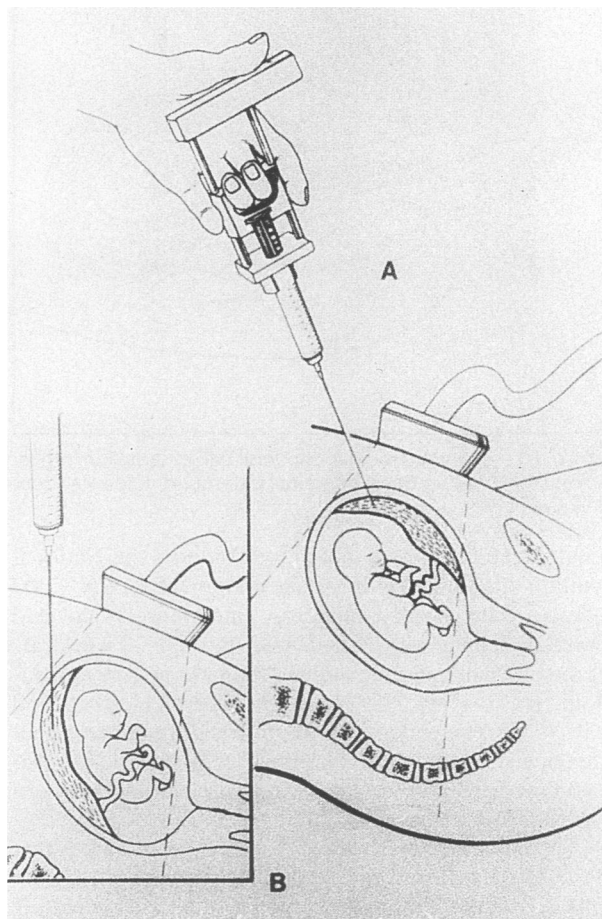
We use a 35-cm, 18-gauge aspiration needle to obtain chorionic villi; the needle is inserted through the posterior vaginal mucosa and uterine wall and into the placenta under continuous ultrasound guidance (Figure 10).<sup>28</sup> Once

the needle tip is in the placenta, the stylet is removed and a 20- or 30-ml syringe containing 4 ml of transport medium is attached. Aspiration is performed in a manner similar to that for transcervical CVS. Optimal specimens weigh 10 to 20 mg.<sup>28</sup> As the safety of transvaginal CVS has not yet been determined, the procedure should not be performed merely in lieu of transcervical or transabdominal sampling.

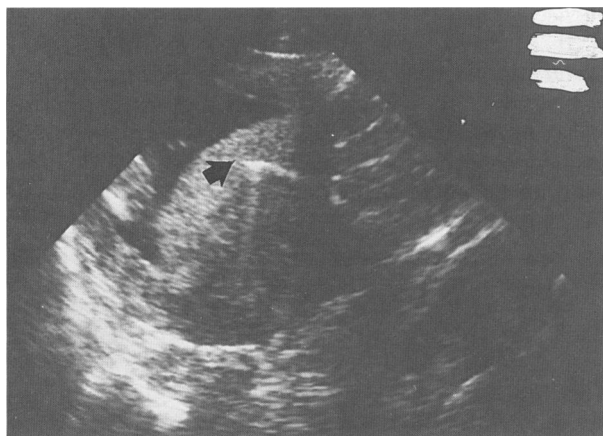
### Safety

Canadian and American multicenter collaborative studies initially reported pregnancy loss rates among women undergoing transcervical CVS to be 0.6% and 0.8% higher, respectively, than among women undergoing second-trimester amniocentesis.<sup>9,10</sup> In neither study were the increased loss rates statistically significant. The Canadian study was randomized whereas the American study involved women who chose either amniocentesis or CVS. In addition, neither study showed a significantly increased incidence of obstetric complications (intrauterine growth retardation, hypertension, abruptio placentae, or premature delivery) among women undergoing transcervical sampling.

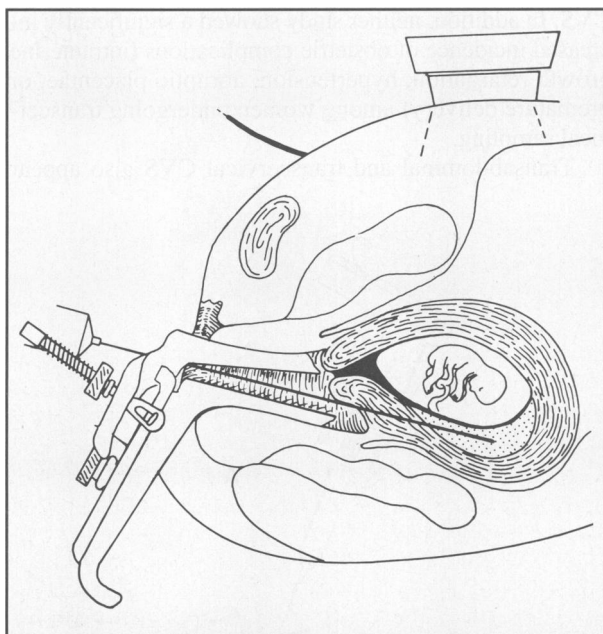
Transabdominal and transcervical CVS also appear



**Figure 8.**—The drawing shows the transabdominal chorionic villus sampling procedure. A, An anterior placenta is sampled. B, Sampling is done on a posterior placenta (from Elias et al<sup>27</sup>).



**Figure 9.**—An ultrasonogram shows a transvaginal insertion of the needle (arrowhead) into the placenta.



**Figure 10.**—The transvaginal chorionic villus sampling procedure is shown, using transabdominal ultrasound guidance (from Shulman et al<sup>28</sup>).

equal in safety. In a collaborative study by the National Institute of Child Health and Development, nearly 4,000 subjects were divided randomly into transcervical and transabdominal groups.<sup>29</sup> Loss rates through 28 weeks after the procedure among women found to be carrying euploid fetuses were 2.5% in the transcervical group and 2.3% in the transabdominal group; this difference was not statistically significant. Brambati and associates also found no differences between transcervical and transabdominal CVS in an Italian randomized trial.<sup>30</sup>

The one major study at odds with these results is that of the Medical Research Council, located primarily in the United Kingdom but including other European centers.<sup>31</sup> The measured outcome was completed pregnancies among women undergoing second-trimester amniocentesis and CVS. The finding of 4.4% fewer completed preg-

nancies in the CVS group was attributed to both more unintended losses and more intended terminations after the detection of fetal cytogenetic abnormalities. The latter finding appears to reflect some inexperience with cytogenetic interpretation of chorionic villi (for example, confined placental mosaicism); the former is more difficult to assess. The experience of the physicians was considerably less than for those participating in the Canadian and American trials; the only prerequisite for participation was the performance of 30 "practice" procedures. Such inexperience may thus have contributed to an increased loss rate among women undergoing CVS in the Medical Research Council trial.

### *Limb Reduction Defects*

Concern has arisen recently about the possible association between limb defects and the sampling procedure. Limb defects involve tissue loss from at least one of the four limbs and are usually divided into two types. Longitudinal defects refer to an interruption along one of the embryologic rays of the hands, arms, feet, or legs. Transverse defects interrupt more than one of the developmental rays of the limbs, frequently resulting in an amputation-like anomaly.

Two centers have reported clusters of limb anomalies in infants whose mothers underwent CVS. Firth and colleagues reported the cases of five infants with transverse terminal limb defects among 289 cases of transabdominal sampling performed between 56 and 66 days' gestation.<sup>32</sup> Burton and colleagues reported the cases of four infants with transverse terminal limb defects among 436 women undergoing both transcervical and transabdominal procedures between 9.5 and 11.5 weeks' gestation.<sup>33</sup>

Although the report of clusters of newborn limb defects in relatively small series of women undergoing CVS is of concern, Jackson of Jefferson Medical College (Philadelphia, Pennsylvania) recently reported that 40 infants with limb reduction defects have been identified among more than 80,000 cases in a multicenter CVS registry, for an incidence of approximately 6 limb defects per 10,000 sampling cases.<sup>34</sup> Froster-Iskenius and Baird have reported a similar incidence of limb defects among all neonates in their analysis of birth registry data from British Columbia.<sup>35</sup> Accordingly, World Health Organization researchers concluded that there is little evidence to suggest a substantive risk of congenital malformations when CVS is performed after the eighth completed gestational week.<sup>34</sup> Chorionic villus sampling should probably not be done routinely before 9.0 weeks' gestation, however, as evidenced by the experience of Brambati from Milan, Italy. Analysis of data from Brambati's highly experienced center demonstrated a small cluster of newborns with limb-reduction defects in cases of very early CVS (7 to 8 weeks' gestation), but no such cluster with cases of traditional sampling (9 to 12 weeks' gestation).<sup>34</sup>

Determining whether the procedure is associated with fetal limb defects will not be an easy task. Many issues serve to preclude a simple statistical analysis, such as gestational age at time of sampling, CVS approach, physi-

cian experience, diagnostic equipment, types of associated limb defects, the interaction of mendelian or polygenic-multifactorial disorders, possible mechanisms of resulting limb defects, and the possible association of other structural defects such as hypoglossia or hypodactylia syndrome. Women considering the procedure should be informed of its possible association with limb defects and of the availability of traditional amniocentesis as an alternative procedure. At the same time, the preponderance of current data does not demonstrate a genuine association between fetal limb reduction and CVS performed by experienced personnel at or after 9 weeks' gestation.

### *Accuracy*

Physicians who care for women undergoing CVS should be aware of the problems associated with the analysis of chorionic villi. Chorionic villi consist of two cell types: cytotrophoblastic cells, which are rapidly dividing cells that are used for direct metaphase analyses, and mesenchymal core cells that are used to initiate cell cultures. Cytotrophoblasts can be directly analyzed within 72 hours, whereas the analysis of mesenchymal core cells requires culture stimulation that usually results in metaphases suitable for cytogenetic analysis in seven to ten days. Both cell types offer advantages and drawbacks for prenatal cytogenetic analysis. Although the direct analysis of cytotrophoblasts can provide rapid results (usually within 24 to 72 hours), the quality of metaphases obtained is usually less than that obtained by culture. Conversely, the culture of mesenchymal core cells provides a better-quality metaphase spread for cytogenetic analysis, although including maternal decidua in the cell culture could lead to an incorrect cytogenetic analysis as a result of maternal cell contamination.

Several obvious problems are associated with the analysis of chorionic villus cells: Cells may not grow, growth may be insufficient for adequate analysis, or rapidly dividing cells may not respond to the laboratory techniques required for metaphase analysis. Fortunately, the combination of cell culture failure and the failure to obtain direct results is uncommon. Maternal cell contamination may lead to an erroneous cytogenetic analysis; the concurrent analysis of uncultured cytotrophoblastic cells and cultured mesenchymal core cells decreases the chance that maternal cell contamination of cultured cells will lead to a discrepant analysis. Careful examination of the chorionic villus specimen under a dissecting microscope also allows chorionic villi to be distinguished from decidua, further decreasing the chance of maternal cell contamination.

Also of concern is the possibility that chromosome abnormalities in chorionic villi do not represent the fetal chromosome complement or, conversely, that a euploid cytogenetic result from CVS does not represent an aneuploid fetal complement. In the US Collaborative Study on CVS, cytogenetic diagnosis (direct, by culture, or both) was achieved in 99.7% of 11,473 cases in which chorionic villi were obtained.<sup>36</sup> No incorrect diagnoses involv-

ing trisomies 21, 18, or 13 were reported. Thirteen unusual aneuploidies (0.11%)—tetraploidy (4 cases) and trisomies of chromosomes 7 (2 cases), 3, 8, 11, 15, 16, 20, and 22—were observed in the direct or culture method, but none by amniotic fluid or fetal tissue cell culture. Mosaic cytogenetic abnormalities were observed with equal frequency in direct and culture preparations in 0.8% of the 11,473 cases but were confirmed as fetal mosaicism more frequently when detected by culture methods rather than direct analyses. Only one incorrect sex prediction was observed in this large series, a 46,XY infant born to a mother whose CVS demonstrated a 46,XX complement; only analysis of cultured chorionic villi was available.

Discrepancies may thus arise between cultured mesenchymal core cells and directly prepared cytotrophoblastic cells. Analysis of the data reported by Ledbetter and co-workers shows the culture method to have a higher degree of diagnostic accuracy than direct analyses, although neither technique is completely reliable.<sup>36</sup> Mosaicism in direct analyses is especially likely not to be confirmed in cultures of mesenchymal core cell or fetal tissue, although recent evidence indicates that such pregnancies may still be at increased risk for an adverse perinatal outcome. The proportion of cases in which direct analyses provide ambiguous cytogenetic results is low (approximately 1%), and most of these ambiguities are resolved by the analysis of cultured mesenchymal core cells. Nonetheless, the direct method can be a useful adjunct to the culture method, in which maternal cell contamination can lead to incorrect sex prediction and, potentially, false-negative diagnostic results.

Despite these caveats, a definitive diagnosis is obtained in almost all cases; only occasionally is amniocentesis required to clarify ambiguous CVS results. Overall, CVS accuracy is comparable to that of amniocentesis, but additional tests (such as amniocentesis) should be considered before acting on nonmosaic rare trisomies and, as in amniotic fluid analyses, polyploidies.

### *Late Chorionic Villus Sampling*

Chorionic villus sampling has also been used for prenatal diagnosis in the second and third trimesters. In most cases, CVS is offered to evaluate fetuses with structural defects in which a more rapid cytogenetic analysis is required for pregnancy management than can be obtained by amniocentesis. Sampling after 12 weeks' gestation is restricted to the transabdominal approach; early experience suggested that the transcervical loss rate was unacceptably high after 12 weeks.

Although late CVS is performed in a manner similar to that for the first-trimester transabdominal procedure, we have found that the aspiration of chorionic villi from second- and third-trimester placentas is somewhat more difficult than aspiration from first-trimester placentas. Accordingly, a more rigorous aspiration technique using an 18-gauge needle is required to obtain suitable specimens.

Diagnostic problems, such as placental mosaicism, encountered in the analysis of chorionic villi obtained



from first-trimester placentas have been encountered in the second and third trimesters as well. We found no diagnostic errors in a small group of 57 chorionic villus samples obtained during the second and third trimesters,<sup>37</sup> and Basaran and colleagues reported no diagnostic errors among 53 specimens evaluated.<sup>38</sup> In addition, Holzgreve and associates reported no immediate or late complications among 73 women undergoing procedures at between 15 and 37 weeks' gestation.<sup>39</sup> Further investigation will be required to determine the safety and efficacy of late sampling.

Late CVS should be considered as an alternative prenatal diagnostic test to percutaneous umbilical blood sampling. At times, it may be the only procedure available to provide prenatal information regarding fetal chromosome complement, as in some cases of oligohydramnios. Although many perinatologists in the United States primarily use umbilical blood sampling rather than late CVS for rapid karyotypic analysis in the second and third trimesters, the choice is less clear-cut elsewhere in the world. Late CVS deserves consideration as an alternative diagnostic procedure for detecting fetal chromosome abnormalities.

### Use and Cost of Prenatal Diagnosis

Amniocentesis and chorionic villus sampling are used primarily by two groups of women: those who are 35 years of age or older at their estimated date of delivery and those who have been found to be at increased risk for fetal neural tube defects or the Down syndrome as a result of maternal serum analyte screening. This screening is performed during the second trimester; accordingly, only amniocentesis is an option for those women who elect invasive prenatal testing after receiving an abnormal screening result. Amniocentesis is available throughout the United States and is performed by many obstetrician-gynecologists and by some family practitioners who provide obstetric services. Conversely, CVS is available at a few centers throughout the United States and is, for the most part, done by a select group of obstetricians with special training in genetics, maternal-fetal medicine, or both. Costs for amniocentesis and CVS vary by geographic location, laboratory, and the specific tests done; however, most insurance plans that cover obstetric services provide reimbursement for either test if the patient is at increased risk for a detectable fetal abnormality.

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